IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 10/627,452	Customer No. 23379
Applicant: Daniel Portnoy and Darren Higgins	Confirmation No. 3443
Filed: Jul 25, 2003	Group Art Unit: 1636
Docket No. B98-039-4	Examiner: Makar, Kimberly A.

Title: *Intracellular Delivery Vehicles*

RESPONSE

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Thank you for the Office Action dated Mar 27, 2007 in the above-referenced case (the "Office Action"). The present Response includes an express abandonment of this application in favor of a contemporaneously filed continuation application. Notwithstanding the express abandonment, Applicant respectfully responds to each of the rejections below, in order to create a clear record.

Improper Final Action

As an initial matter, Applicant notes that the Office Action was improperly designated as a "final" Office Action. The Office Action applies a new ground of rejection not necessitated by any amendment made by Applicant; accordingly, the subject action is properly construed to be non-final.

In particular, the Action offers a new ground of rejection under 35USC103(a) over Darji et al. (1997) in view of Powell et al. (US Pat No 5,877,159) and Darji et al. (1995) and Dietrich et al. (1998). This rejection was not present in the prior Office Action (dated Jul 12, 2006).

Instead, this prior Office Action made a facially improper rejection under 35USC102(e) as anticipated by Darji et al. (1997), and a rejection under 35USC103(a) over Powell et al. (US Pat No 5,877,159) in view of Darji et al. (1995) and further view of Dietrich et al. (1998). Applicant rebutted these rejections with argument and they were removed in favor of the new 35USC103(a) rejection. This new ground of rejection was responsive not to any amendment, but to Applicants rebuttal of the previously applied prior art rejections.

35USC112, first paragraph (enablement)

The enablement requirement provides that the specification enable one of ordinary skill in the art to practice the invention without undue experimentation. The claims addressed in the Office Action are directed to a method comprising the step of introducing a foreign antigenic (claim 13) or therapeutic (claim 26) agent into a human cell by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell, wherein an immune (claim 13) or physiological response (claim 26) is generated.

The Specification teaches the introduction of a variety of foreign agents for a variety of purposes (e.g. p.4, line 19 - p.6, line 3), including antigenic and therapeutic agents. The Specification teaches use of a variety of nonvirulent bacteria (e.g. p.6, lines 4-20) and target cells (e.g. p.6, line 21 – p.7, line 12). The specification teaches a variety of effective routes of in vivo and ex vivo administration depending on the nature of the foreign agent (e.g. p.7, line 13 - p.8, line 14). In addition, the specification provides numerous demonstrative examples of the method as applied to numerous foreign therapeutic agents (note that in some embodiments, an antigenic agent will suffice as a therapeutic agent; therapeutic agents include prophylactics such as immunizations, e.g. p.4, lines 21-22) and a variety of target cells in vivo and ex vivo (e.g. p.8, line 15 - p.10, line 11).

The application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation. In fact, following the teachings of this disclosure without resorting to any undue experimentation, persons skilled in the art have implemented the claimed method to generate an anti-tumour response and to deliver antigens in

vivo (e.g. Radford et al., Gene Therapy 2002, 9, 1455-63; Bouwer et al. PNAS 2006, 103, 5102-7; both attached).

For good measure, we have of record undisputed, affirmative evidence in the form of an expert declaration averring to the foregoing. Accordingly, the uncontroverted evidence of record demonstrates that the application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation.

35USC103(a)

The cited references (Darji et al. (1997, Cell 1997, 91, 765-75) in view of Powell et al. (US Pat No 5,877,159), Darji et al. (1995, J Biotech 43, 205-212) and Dietrich et al., 1998, Nat Biotech 16, 181-85) do not teach or suggest the claimed invention.

The claims addressed in the Office Action recite a method comprising the step of introducing a foreign antigenic (claim 13) or therapeutic (claim 26) agent into a human cell by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell, wherein an immune (claim 13) or physiological response (claim 26) is generated.

Darji (1997) describe oral immunization of mice with attenuated *typhimurium aroaA* that is transformed with eukaryotic expression vectors encoding truncated variants of ActA and listeriolysin (two virulence factors of Listeria monocytogenes). Darji, Abstract. The listeriolysin immunogen was a nonsecreted and nonhemolytic fragment consisting of amino acids 26-482 (e.g. Darji, p.766, col.1, lines 27-29) of the native 529 amino acids (e.g. Lety et al., Microbiology 149 (2003), 1249-1255, attached).

The pending claims require a promoter that expresses the cytolysin in the bacterium. Darji et al. teach the opposite: that the cytolysin is expressed not by the bacterium, but by the targeted eukaryotic cell (e.g. Darji, p.766, col.1, lines 5-7, 20-21 and 41-51). Darji et al. require and exclusively teach a eukaryotic promoter, and can not and do not suggest, alone or in combination with any or all of the secondary references, the express limitations of our claim that requires a promoter which expresses the cytolysin in the bacterium.

Furthermore, all of the pending claims require expression of a functional cytolysin. Darji (1997). teach the opposite: their listeriolysin variant is non-functional (non-lytic), double-truncation (e.g. Darji, p.766, col.1, lines 27-29) – and necessarily so, because their protein is used not for delivery (it is not even expressed until after integration into the eukaryotic cell), but as an immunogen (e.g. Darji, p.766, col.1, lines 23-31; p.768, para. bridging cols.1-2; Fig.4).

Powell et al. use live, invasive bacteria to enter animal cells and deliver eukaryotic expression cassettes for expression in said animal cells (col.6, lines 48-62). In addition to naturally invasive bacteria, Powell et al. propose that noninvasive bacteria could be genetically engineered to enter the cytoplasm of animal cells, using various invasive proteins, including listeriolysin O of Listeria (col.8, lines 36-42; col.10, line 35 – col.11, line 11). Powell et al. require and exclusively teach that live bacteria enter the cytosol or nucleus of the target animal cell (e.g. col.8, lines 36-42; col.10, lines 45-46 and 50-51; line 11; col.13, lines 64-65; col.14, liens 5-6 and 14-15).

Dietrich et al. describe delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide Listeria monocytogenes. Dietrich's method uses a Listeria monocytogenes bacterium having attenuated virulence and expressing a bacteriophage lysis gene (PLY118 – the suicide gene) under the L. monocytogenes *actA* promoter, which is activated in the cytosol of the infected cell; Dietrich, p.181, col.2, lines 17-18. Dietrich's bacteria are specifically designed to escape to the cytosol of the infected cell where the suicide gene can be activated; Dietrich, p.181, col.1, line 24; p.184, col.2, lines 7-9. The antigens (GFP, CAT (chloramphenicol acetyl transferase), and OVA257-264 (T cell-reactive H-2K epitope) are then expressed by the host macrophage through a eukaryotic promoter (Dietrich, p.183, col.1-2).

The Action proposes imposing Dietrich's PLY118 gene[1] to "add to the safety of the attenuated L. monocytogenes".... But Powell et al. use live, invasive bacteria to enter animal

[1] PLY118 is not even a cytolysin – it does not lyse any cellular membranes. It is a peptidase endolysin – it is secreted through the membrane of the bacteria and attacks a component of the surrounding bacterial wall; see Dietrich et al. p.182, col.1, line 1, where Dietrich cites Loessner et al., 1995, Mol Microbiol 16, 1231-41, abstract enclosed. Loessner explains that PLY118 is a cell wall lytic enzyme which specifically cleaves between the L-alanine and D-glutamate residues of listerial peptidoglycan.

cells and deliver eukaryotic expression cassettes for expression in said animal cells (supra), so adding a PLY118 gene would only yield the method of Dietrich.

Absent hindsight provided by the present invention, there is no motivation to combine the cited references. Moreover, no such combination could teach or suggest the present claims. Specifically, The cited references have not been shown to provide any suggestion for introducing a foreign agent into a human cell using non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell.

The Examiner is invited to call the undersigned with any suggestions for amending the claims or further clarifying any of the foregoing. Please charge any required fees to our Dep. Acct. No.19-0750 (order B98-039-4).

Respectfully submitted,
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Encl. PTO/SB/24

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